A field test for frequency-dependent selection on mimetic colour patterns in *Heliconius* butterflies

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SUPPLEMENTARY MATERIAL

Model construction

Since it is well known that birds see a wider colour spectrum than humans (Cuthill, 2006), we calibrated the colours printed on the models so that they stimulate the photoreceptors of potential bird predators in a manner comparable to the real wing colours. The method followed is similar to that of Cuthill *et al.* (2005) and Stevens *et al.* (2007). In brief, the reflectance spectra of each colour patch on real wings was measured with an Ocean Optics USB4000 spectrometer (Dunedin, FL), with illumination by a PX-2 pulsed Xenon lamp with a narrow-ended (1/8″) probe, held at a constant distance and a 45 degree angle to the butterfly wings. Three replicate measurements were taken per colour patch, in each of four specimens selected per colour pattern. Patches of the same colour on fore- and hindwings were measured separately. Since the reflectance spectra measurements were highly consistent within each colour patch, between patches of the same colour, and even between individuals with either the same or different colour pattern, the averages of all measurements per colour were used for further analysis (Figure S1). The next step was to calculate the photon catch values (as Endler & Mielke, 2005) for each of the four single cone types (used in colour vision) and the double cones (used in achromatic vision) for a typical insectivorous bird (a blue tit, *Cyanistes caeruleus*), using data from Hart *et al.* (2000) and a standard D65 irradiance spectrum. Although neither irradiance spectra from the field locations nor sensitivity data for the real species of bird predators were available, the level of error associated with those factors should be relatively small as different irradiance spectra have little effect on the outputs of most visual models in terrestrial systems (e.g. Langmore *et al.*, 2009) and birds seem to have relatively constrained visual sensitivity across species (Cuthill, 2006). The colours to be printed in the final models were calculated by printing a range of colour values around those observed on real wings and measuring the photon catch values produced. An iterative process was used, whereby this procedure was repeated, changing the closest colours slightly until photon catches were as close as possible to those of real wings (see main text, Figure 3). Final colours were selected based on the criteria that when printed they produced photon catch values for each cone type that fell within the range of values for the corresponding colour patches on the real butterflies (as with past work on camouflage: e.g. Cuthill *et al.*, 2005, and mimicry: Merrill *et al*. 2012). The calibrated colours were used to replace the colours on digital images of real wings (see main text), using Adobe Photoshop®, and these final print outs were used to build the models for the experiment.

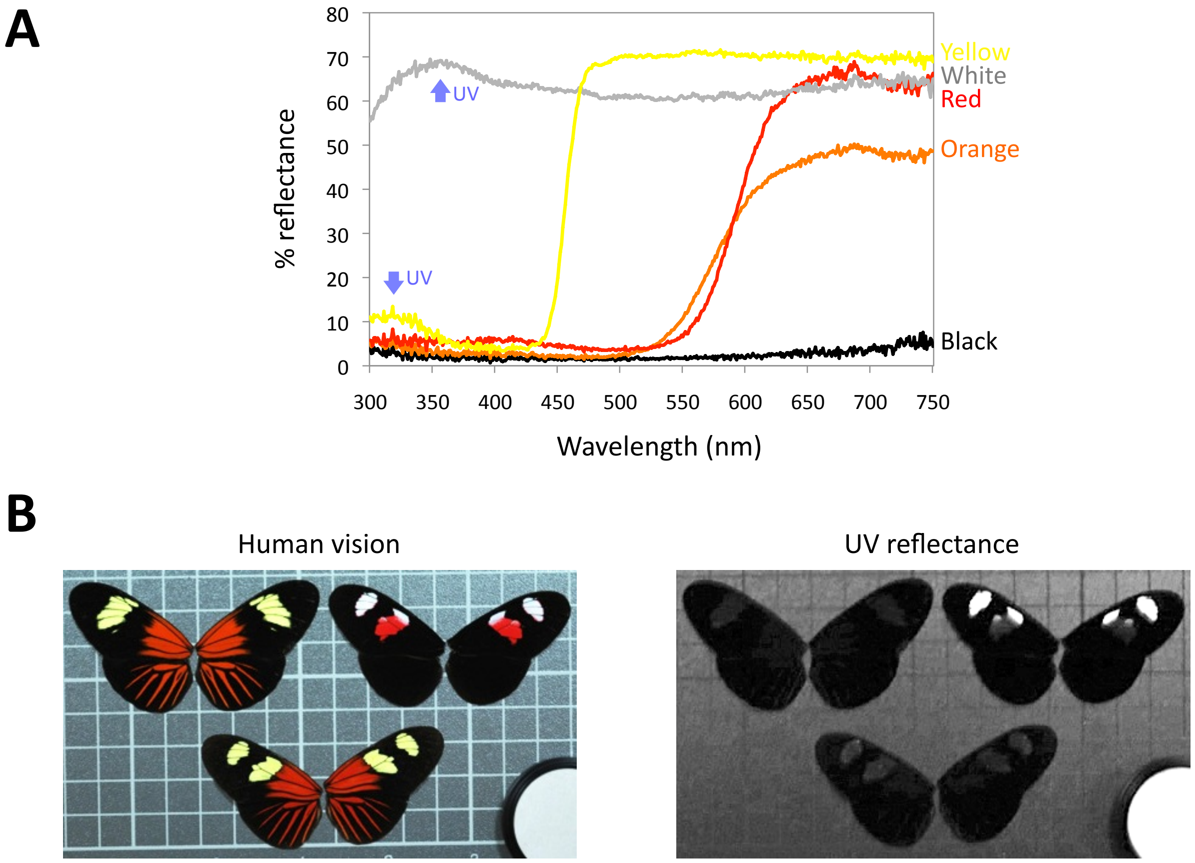
Control of potentially confounding factors

Table S1 lists all potentially confounding factors and the strategies applied to control for their potential effects during the experiment to test for frequency-dependent selection.

Table S1. Potentially confounding factors and applied controls.

Potentially confounding factor	**Applied control**
Observer bias: When marks were weak some observers could consider a model as marked whereas others as intact.	Sets of models were assigned randomly to observers, making sure that no single observer checked the same set of models twice in a row. This way, any observer bias is expected to equally affect all observations across all patterns and all zones.
Seasonality: The experiment lasted for three months (July-September 2010). During this period, seasonal variations in rainfall and/or temperature could affect predation patterns.	Experimental trials were alternated between zones. For instance, the first trial was performed in one of the parental zones, the second trial in the hybrid zone, the third one in the other parental zone, and the fourth one back in the first zone. Any possible effect of seasonality in a particular zone is expected to equally affect all tested colour patterns.
Microhabitat heterogeneity: Predation patterns could vary depending on microhabitat characteristics such as luminosity or vegetation coverage. For instance, predation inside a covered forest is likely to differ from that along a forest edge or a river shore.	Models of each tested colour pattern were placed one after the other along the sampled transects. This way it was ensured that all three patterns were equally exposed to all habitats where the experiment was carried out. Any effect due to microhabitat differences is expected to affect all colour patterns equally.
Reutilization of models: It was not practical to use new paper wings for each experimental trial. Therefore, paper wings were reused up to three times. Wearing of paper wings could affect the probability of the models being attacked.	The same proportion of new versus reused paper wings was used for all colour patterns in all studied zones. Any affect of wings wearing is expected to equally affect all colour patterns in all zones.

Figure S1


A
80
70
60
50
40
30
20
10
0
% reflectance
UV
UV
Yellow
White
Red
Orange
Black
300
350
400
450
500
550
600
650
700
750
Wavelength (nm)
B
Human vision
UV reflectance

SUPPLEMENTARY FIGURE LEGEND

Figure S1 (A) Average reflectance spectra of colour patches on real wings in the studied phenotypes of *Heliconius erato*. Notice the UV reflectance peak on the white patches of the highlands race, and to a lesser extent on the yellow patches of the hybrid and lowlands race. (B) Photos of wings illustrating the UV reflectance on the white patches of the highlands race.